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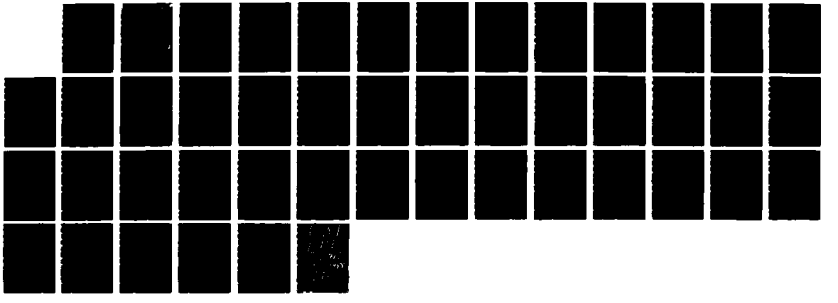

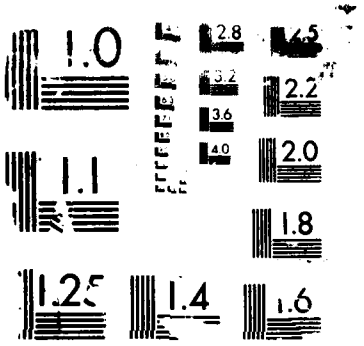

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Studies of the Biology of Phleboviruses in Sandflies

Annual/Final Report

Robert B. Tesh, M.D.

31 August 1987

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SUMMARY

The objectives of this research project were to study the behavior of selected phleboviruses in their sandfly vectors and to determine the mechanism by which these agents are maintained in nature. Initially, a number of sandfly colonies were established in the laboratory. These insects were then experimentally infected with a number of different phleboviruses in order to study development of the agents in the insects and to determine whether or not the viruses were transovarially transmitted in their sandfly vectors. All of the viruses that were tested, replicated in sandflies after inoculation; but these insects were more difficult to infect orally. Many of the viruses were vertically transmitted, with transovarial transmission rates as high as 90% in the F_1 progeny of infected females. Results of these studies suggest that phleboviruses are maintained in nature by two mechanisms: transovarial (vertical) transmission in their insect vector and period amplification in a vertebrate-insect cycle.

Studies were also done in hamsters to determine the viremia and immune response following sequential phlebovirus infection. This work indicated that a brief period of viremia occurred with each phlebovirus infection and that the antibody response, as measured by plaque reduction neutralization test, was monotypic even after several consecutive infections.

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Foreword

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 86-23, Revised 1985).

A. History of the Project

The objective of this research project was to study the biology and transovarial transmission of phleboviruses in sand flies. The project began on 1 September 1980, and it ended on 18 May 1987. During this period, it was funded by three different contracts and one grant (listed below). Although the mechanism of funding changed several times, the overall objective of the project did not change, nor did the personnel working on it.

Source of funding	Title	Duration
DAMD17-80-C-0178	Studies on the Transovarial Transmission of Phlebotomus Fever Viruses in Sandflies	1 Sept.1980-30 Sept.1982
DAMD17-83-C-3002	Studies on the Biology of Phleboviruses in Sandflies	1 Oct. 1982-30 Sept.1983
DAMD17-83-G-9561	Studies on the Biology of Phleboviruses in Sandflies	1 Oct. 1983-30 Sept.1984
DAMD17-85-C-5023	Studies on the Biology of Phleboviruses in Sandflies	10 Dec. 1984-18 May 1987

B. Sandfly Colonization

During the first 16 months of the project (Sept. 1980 - Dec. 1981), efforts were focused primarily on establishing laboratory colonies of phlebotomine sandflies. This work took much longer than anticipated; and, in retrospect, our original timetable was unrealistic. We found, as many other investigators before us have found, that sandflies are difficult to rear in captivity. These insects are much less prolific than mosquitoes and each generation takes approximately 6 to 8 weeks to develop. Furthermore, the daily care of sandflies is extremely labor intensive. Therefore, new techniques for rearing the insects had to be developed.[1] For these reasons, it was not until December of 1981 that we finally had sandfly colonies of sufficient size and productivity to actually begin experimental studies. By the end of the project, we had refined our mass-rearing techniques to the point where we could produce thousands of insects per week; the only real limitation is the number of hours required to maintain the colonies.

During the 6½ years of the project, 11 species of sandflies were colonized. A list of the successful species and their origin is given below:

Phlebotomus papatasi (India)	*Lutzomyia longipalpis* (Brazil)
Phlebotomus papatasi (Israel)	*Lutzomyia longipalpis* (Colombia)
Phlebotomus papatasi (Egypt)	*Lutzomyia gomezi* (Colombia)
Phlebotomus argentipes (India)	*Lutzomyia seranna* (Colombia)
Phlebotomus perniciosus (Italy)	*Lutzomyia abonnenci* (Panama)
Phlebotomus martini (Kenya)	*Lutzomyia anthophora* (U.S.A.)
	Lutzomyia columbiana (Colombia)

Some of these species were obtained from already established laboratory colonies (i.e. Ph. papatasi (Israel) and Ph. martini (Kenya), but others were started from field-collected material. Our experience with sandflies suggests that, in initiating a new colony, a minimum critical number of the insects is needed. Successful mating and insemination of female sandflies appears to be directly related to the number of males and females caged together. Single pair matings rarely yield fertile eggs; and even when 10 or 20 flies are caged together, the percentage of viable eggs that are produced is low.

Another observation that we have made is that sandflies which normally inhabit drier regions of the world (i.e. Ph. papatasi, Lu. longipalpis, Lu. anthophora) are much easier to maintain in the laboratory than species from humid, tropical regions. We tried repeatedly to colonize Lu. trapidoi, Lu. hartmanni and Lu. flaviscutellata without success. All inhabit humid tropical forests in the New World. None of these species could be maintained for more than 1 or 2 generations in the laboratory, despite the fact that we started with several hundred eggs or larvae initially. Species from drier areas just seem to be much stronger in general; they also survive much better after intrathoracic inoculation.

C. Sandfly Cell Cultures

Two sandfly cell cultures were developed as a result of this project. These were the LL-5 cell line, started from eggs of Lutzomyia longipalplis, and the PP-9 cell line, started from eggs of Phlebotomus papatasi. The technique and culture medium used to start these cell lines has been described previously.[2] Initially, we attempted to start cell cultures from gravid females and from larvae; but the adult tissues did not grow, and larval fragments inevitably became contaminated with bacteria or fungi because of our inability to sterilize the larval gut prior to culture. We finally used surface sterilized, embryonating eggs to initiate the cultures. The disadvantage of eggs is that they contain a variety of cell types, and cultures started from eggs usually contain several cell types of unknown origin.

The ability of the LL-5 and PP-9 cell lines to support arbovirus growth was investigated. Susceptibility of the LL-5 cells to 29 different arboviruses was tested. The viruses examined included representatives of the genera Vesiculovirus, Orbivirus, Flavivirus, Alphavirus, Bunyavirus and Phlebovirus. These results have already been published[2] but are summarized again in Table 1. Only 13 of the viruses multiplied in the LL-5 cells; however, many of the viruses examined were mosquito-associated agents and might not be exptectd to grow in sandfly cells. Surprisingly, however, most of the phleboviruses did not replicate. Of the arboviruses which did grow in the LL-5 cells, only Changuinola virus, a sandfly-associated orbivirus, produced moderate cytopathic effect; the remainder produced non-cytopathic persistant infections.

Fewer arboviruses were tested in the PP-9 cell line, but the results were similar. Most of the phleboviruses, including Naples and Sicilian sandfly fever viruses which have been isolated from naturally infected Ph. papatasi, failed to grow in the PP-9 cells. Since the origin of both cell lines was confirmed by isozyme analysis,[3] one can only conclude that the cell types present in the two cultures do not support the growth of most phleboviruses.

D. Ovarian Development in Sandflies

Because relatively little is known about the reproductive biology of phlebotomine sandflies and since this project is primarily concerned with the transovarial transmission of sandfly-associated viruses, investigations were done on ovarian development in these insects. Results of this work have already been published,[4] but are summarized below.

Ovaries of laboratory-reared Ph. papatasi, Lu. longipalpis and Lu. anthophora were examined to determine rates of autogenous and anautogenous oocyte development and to describe morphological changes in ovarioles before and after ovulation. Female Ph. papatasi, having origins in India and Israel, produced eggs autogenously within 6 days after eclosion. If suitable hosts were available, these insects also ingested blood during the first gonotrophic cycle. Females of both Lutzomyia species developed eggs 3-6 days after a blood meal. Dissections of 491 Ph. papatasi females, collected from human dwellings and animal shelters in Iran, revealed predominately nulliparous specimens (94%) with oocytes at or beyond stage III in 161 individuals (33%). Of these, 87 (54%) had vertebrate blood in their midguts. Follicular relics were observed in both colonized and field-collected females.

Our observations support previous reports[5,6] that some sandfly species will take blood repeatedly during a single gonotrophic cycle, if suitable vertebrate hosts are available. This characteristic (multiple feedings on different hosts) increases their vector potential. Similarly, the ability to produce eggs autogenously would also insure the survival of a transovarially transmitted pathogen in the absence of a susceptible vertebrate host for the insect to feed upon.

E. Experimental Infection of Sandflies with Phleboviruses

Initially, we compared the susceptibility of several sandfly species to oral and parenteral infection with different phleboviruses. Most of this work has already been published,[7-10] but the results are summarized in Tables 2-23. Results of these studies indicated that all of the phleboviruses, which were tested, replicated to some degree in sandflies after intrathoracic inoculation. The viruses replicated in both vector and non-vector species. In contrast, sandflies were difficult to infect orally. Even in natural virus-vector combinations, such as Naples in Ph. papatasi (Table 18) and Arbia in Ph. perniciosus (Table 17), the viruses did not survive more than 2 or 3 days after ingestion. The only viruses which did replicate in sandflies after ingestion were Toscana virus in Ph. perniciosus (Table 21), Arboledas virus in Lu. gomezi (Table 23), and possibly Gabek Forest virus in Ph. papatasi (Table 22). The reason(s) for this refractoriness to oral infection is unknown. It is noteworthy, however, that similar results were obtained with Carajas virus, a sandfly associated rhabdovirus of the vesicular stomatitis serogroup (Table 24).[11]

F. Transovarial Transmission of Phleboviruses by Sandflies

The next group of experiments that were done were designed to investigate the frequency with which phleboviruses were vertically transmitted in sandflies. Because of our difficulty in infecting these insects orally and the

fact that most laboratory-reared female sandflies die during or shortly after oviposition,[1,4] the flies were infected by intrathoracic inoculation. A total of 14 different phleboviruses were tested for transovarial transmission in one or more sandfly species. Results are summarized in Table 25. Transovarial transmission rates (percentage of F_1 progeny infected) varied from 0 to 81%, depending on the virus-vector combination used. The results of these experiments also indicated interspecies differences in the ability of sandflies to vertically transmit a given phlebovirus. For example, Ph. perniciosus females, infected with Toscana virus, transmitted the virus to 40% of their F_1 progeny, while none of the offspring of infected female Ph. papatasi or Lu. longipalpis contained virus (Table 25).

In another series of experiments, an attempt was made to see how long Toscana virus could be maintained in Ph. perniciosus by transovarial transmission alone. Initially, 100 Ph. perniciosus females were inoculated intrathoracically with Toscana virus. Five days after inoculation, the infected females were fed on a clean hamster, and their combined eggs from the first ovarian cycle were collected. After hatching, the F_1 larvae were reared to adults, which were allowed to mate randomly and then to feed on another clean hamster. Their first ovarian cycle eggs (F_2 generation) were subsequently collected and the entire process was repeated again for 13 consecutive generations. A sample of adults of both sexes from each generation was frozen at -70°C and saved for subsequent virus assay. Each Ph. perniciosus generation took approximately 40 days to complete its life cycle; the entire time period from initial infection of the parental generation to the emergence of the F_{13} generation was approximately 23 months. A pedigree of the infected line is given in Table 26.

Two important facts should be noted about Table 26. First, only the parental generation was experimentally infected by inoculation; in all subsequent generations, infection resulted from transovarial virus transmission. Second, at the F_2, F_4 and F_{12} generations, only eggs from infected females were used to start the next generation. In the other generations (F_1, F_3, F_5-F_{11}), eggs from all females (infected and non-infected) were pooled to start the next generation.

Table 26 shows the number of progeny sampled and the percentage of offspring infected in each generation. In the parental generation, 100% of the inoculated females contained virus when tested after oviposition. Forty percent of their progeny (F_1 generation) were transovarially infected and contained virus. From the data shown in Table 26, it can be seen that Toscana virus was maintained in the infected Ph. perniciosus line for 13 consecutive generations (approximately 23 months). It is also apparent that the percentage of transovarially infected flies did not remain constant but tended to decrease in subsequent unselected generations. For example, from the F_5 to the F_{12} generation, the percentage of transovarially infected insects declined from 86.0 to 5.8%. Only after selection (pooling eggs from virus-positive females) at the F_2, F_4, and F_{12} generations did the infection rate in the next generation show a significant increase.

In order to determine if there were differences in the filial infection rates among various females, 15 Ph. perniciosus from the parental generation

were placed in individual oviposition containers and their eggs were collected. Their F_1 offspring were then reared to adults and tested individually for virus. Results are shown in Table 27. All of the females in this group transmitted virus to some of their progeny, but in no case were all of the offspring infected. Filial infection rates in the progeny from females in this experiment varied from 37.5 to 92.3%. This probably accounts for the steady decline in Toscana virus infection rates observed from the F_5 to the F_{12} generations (Table 26). With filial infection rates less than 100%, the percentage of uninfected flies in each subsequent generation increased, thereby steadily lowering the virus infection rates.

Two experiments were done to determine what effect, if any, prolonged transovarial passage of the virus might have on its pathogenicity for vertebrates. In the first experiment, four newborn mice were placed for several hours in a cage with about 100 F_7 generation Ph. perniciosus females from the chronically infected line (Table 26). Three of these mice became sick on the fourth day after exposure to the flies, and Toscana virus was subsequently recovered from their brains. This indicated that the transovarially infected females were still capable of transmitting the virus by bite and also that the virus had not lost its pathogenicity for mice after transovarial passage through seven consecutive generations of sandflies.

In a second experiment, the animal pathogenicity of the original Toscana pool, used to infect the parental generation, was compared to that of a virus suspension prepared from an infected F_{13} generation female sandfly (Table 1). Both virus stocks were titrated simultaneously in newborn mice and in Vero cells. Virus titers in the mouse and Vero assay systems were as follows: the original Toscana virus stock, $10^{6.5}$ $SMLD_{50}$ and $10^{5.8}$ PFU per ml, respectively; and the F_{13} virus, $10^{3.9}$ $SMLD_{50}$ and $10^{3.9}$ PFU per ml, respectively. Although the titer of the mouse brain stock was considerably higher than that of the sandfly suspension, these data suggest that the mouse pathogenicity of the virus, compared to its infectivity for Vero cells, did not change significantly during prolonged (23 months) transovarial passage in Ph. perniciosus.

Studies were also done to determine if detectable genetic changes had occurred in the Toscana virus strain maintained by vertical transmission in the chronically infected Ph. perniciosus line (Table 26). T1 ribonuclease fingerprint analysis of RNA genomes was done on Toscana virus isolates from the original parental, F_2, F_7 and F_{12} generations.[21] No virus RNA genome changes were detected during transovarial transmission of the virus through 12 consecutive sandfly generations (a period of almost 2 years). These results indicate that although RNA viruses can exhibit high rates of mutational change under a variety of conditions, Toscana virus RNA genomes are apparently maintained in a stable manner during repeated transovarial virus transmission in the natural insect host.

An attempt was also made to determine whether the efficiency of transovarial passage of Toscana virus changed after prolonged vertical transmission in sandflies. In this experiment, Ph. perniciosus females from an uninfected laboratory colony were divided into two groups. One group was inoculated with the original Toscana mouse brain stock, used to infect the

parental generation; the other was inoculated with a Toscana virus suspension, prepared by homogenizing an infected F_9 generation female (Table 26). The percentage of infected F_1 progeny from the two groups was 37.7% (29/77) and 69.8% (67/96), respectively. Although the transovarial transmission rate was higher with the vertically passaged virus, it still did not approach 100%. Since studies[14-16] with several other viruses have suggested that transovarially infected insects may be slower in their development, we examined what effect Toscana virus infection had on the developmental rate of Ph. perniciosus larvae and pupae. In this study, the F_7 generation of the chronically infected line (Table 26) was used. Their parents (the F_6 generation females) were all fed at the same time. As shown in Table 28, the F_7 generation adults emerged over a 24-day period. Overall, 62.9% of these flies were infected. However, the infection rate among the emerging adults remained fairly constant during the 24-day period, indicating that Toscana virus had little or no effect on the insects' rate of eclosion. Results of this experiment also indicate that males were infected at almost the same rate as females.

The results of the experimental studies shown in Table 26 indicate that Toscana virus can be maintained in Ph. perniciosus solely by transovarial transmission for several consecutive generations, without significant biological change. However, the data (Tables 26 and 27) also imply that the virus cannot be maintained indefinitely in sandflies by vertical transmission alone. This observation plus the fact that Ph. perniciosus can be infected orally with Toscana virus (Table 21) suggest that amplification in a vertebrate-insect cycle must occassionally occur in nature in order for the virus to survive. Thus, transovarial passage of Toscana virus would appear to be a safety mechanism which allows the virus to survive during adverse climatic conditions (i.e. winter) or in the absence of a susceptible vertebrate host. Because of the relatively short life span and limited flight range of sand flies, the virus may have to use both maintenance cycles to insure its survival.

A third possible maintenance mechanism, which would amplify the virus in nature, is venereal transmission by transovarially infected male sandflies to non-infected females. This phenomenon has been demonstrated experimentally with two other arboviruses in mosquitoes and ticks[18-20]. However, to date we have been unable to show venereal transmission of Toscana virus in Ph. perniciosus in the laboratory.

G. Studies of the Viremia and Immune Response with Sequential Phlebovirus Infections

As part of this project, a series of experiments were also done to determine the level of viremia and immune response occurring with sequential phlebovirus infections. Four groups of hamsters were infected sequentially with various combinations of Arumowot, Chagres and Gabek Forest viruses. Following each infection, the survival, level of viremia, and immune response of the animals were monitored.

Experiment 1

In the first experiment (Table 29), six normal hamsters were given Gabek Forest virus as their primary infection. On the second and third days post-

inoculation, all of the animals had high levels of virus in their blood ($10^{7.6}$ - $10^{9.6}$ PFU/ml). By the fourth day, all of the hamsters were dead. In these non-immune animals, Gabek Forest virus produced a fulminating and uniformly fatal disease.

Experiment 2

The six hamsters in this experiment were infected sequentially at 3-4 week intervals with Arumowot, Chagres and Gabek Forest viruses. The levels of viremia and neutralizing antibody titers developing in the animals after each infection are shown in Table 30. Primary Arumowot virus infection produced a viremia of 3 to 4 days duration, with maximum virus titers ($10^{5.0}$ - $10^{7.0}$ PFU/ml) occurring on the second and third days post-inoculation. All of the animals survived, and the neutralizing antibody response was quite specific.

Three to four weeks later, when the hamsters were given their second infection with Chagres virus, they again developed viremia. Although the level and duration of Chagres viremia was less than that observed during Arumowot virus infection, the level of virus present in the blood after the second infection was not significantly different from the viremia developed by hamsters whose primary infection was with Chagres virus (Experiment 3 - Table 31). These data suggest that previous infection with Arumowot virus did not modify the viremia developing in the animals after Chagres infection. The titer of Chagres virus neutralizing antibodies present in the hamsters' convalescent sera after the second infection also indicates that their immune response was not altered. It is interesting that three of the hamsters in this experiment (animal #2229, 2235 and 2261) showed a rise in neutralizing antibody titers to Arumowot virus after the second infection, suggesting that Chagres virus had a booster effect. It is also noteworthy, that even after the second phlebovirus infection, none of the hamsters had detectable levels of neutralizing antibodies to Gabek Forest virus.

Of the six hamsters which were inoculated with Gabek Forest virus in the third phase of this experiment, only two died. Hamster #2235 died on the fourth day after infection. Unfortunately no blood sample was obtained prior to death, but it seems probable that this animal developed a fulminating infection. Hamster #2234 was found dead two weeks after inoculation with Gabek Forest virus, and it most likely died of other causes. The fact that no virus was detectable in the animal's blood on the fourth day of infection supports this view. Of the remaining four animals (#2229, #2231, #2261 and #2262), virus was only detected in hamster #2261. The level of viremia in this animal was considerably less than the levels observed in Gabek Forest-infected hamsters in Experiment 1 (Table 29). No virus was detected in the blood of the other three animals (#2229, 2231 and 2262); however, they were only sampled on one or two days after infection and a transient viremia could easily have been missed. Nonetheless, all of the surviving animals in this experiment had neutralizing antibodies against Gabek Forest in their convalescent sera, indicating that they had been infected. The latter data imply that immunity to Arumowot and Chagres viruses somehow modified the third (Gabek Forest) infection.

Experiment 3

The eight hamsters in this experiment (Table 31) were infected sequentially with Chagres, Arumowot and Gabek Forest viruses, respectively. The viremias developed by the animals during their primary Chagres infection were similar to those observed in hamsters in Experiment 2 (Table 30, Second infection). Three weeks after their primary infection, the animals only had neutralizing antibodies to Chagres virus.

During their second phlebovirus (Arumowot) infection, the viremias developed by these Chagres -immune animals were similar to those observed in hamsters initially infected with Arumowot virus (Tables 30 and 32). These data again indicate that the previous Chagres virus infection did not modify the second infection with Arumowot virus. Similarly, all of the hamsters had neutralizing antibodies to Arumowot virus in their convalescent sera (Table 30), demonstrating a normal immune response to the second infection (Hamster #2236 died before a second convalescent serum could be obtained). It is noteworthy that none of the surviving hamsters had detectable levels of Gabek Forest neutralizing antibodies in their second convalescent sera.

Upon challenge with Gabek Forest virus (third infection), the remaining seven hamsters in this group all developed viremia. Hamster #2237 developed a fulminating Gabek Forest virus infection and died on the fourth day; hamsters #2263 and #2264 had similar fatal infections and died on day 5. The other four animals (#2238, #2241, #2265 and #2270) also developed viremias, but they survived infection and subsequently developed Gabek Forest virus neutralizing antibodies in their convalescent sera. Although the hamsters in this experiment were only bled once or twice, the data (Table 30) suggest that viremia levels in challenged animals were lower than in hamsters with primary infection.

Experiment 4

The hamsters in this experiment (Table 32) were initially infected with Arumowot virus. Three weeks later, they were challenged with Gabek Forest virus. The purpose of the experiment was to determine whether a single phlebovirus infection would protect the animals from the lethal effects of Gabek Forest virus.

The viremia and immune response of the eight hamsters to initial Arumowot virus infection were similar to those observed with the same agent in Experiments 2 and 3 (Tables 30 and 31). When challenged with Gabek Forest virus three to four weeks later, all of the hamsters developed viremia, and six of the eight (75%) animals died. From the data given in Table 32, it can be seen that levels of viremia in the six dying hamsters were higher than levels detected in the two survivors (#2256 and #2259). It is also interesting that hamsters #2239 and #2257 died on the sixth and seventh days after infection, respectively, suggesting that their immunity to Arumowot provided partial protection and somehow delayed the lethal effects of Gabek Forest virus infection. It is also noteworthy that although the two surviving animals in this experiment (#2256 and #2259) developed Gabek Forest neutralizing antibodies after infection, their second convalescent sera had no detectable neutralizing activity against Chagres virus.

In summary, all of the agents produced viremia in the hamsters, regardless of the order of their administration. The antibody response, as measured by plaque reduction neutralization test, was monotypic even after two consecutive phlebovirus infections. Arumowot and Chagres viruses produced non-fatal infections in adult hamsters, which were characterized by viremia of several days duration and subsequent antibody formation. In contrast, Gabek Forest virus produced a fulminating and rapidly fatal disease in phlebovirus non-immune animals. In hamsters previously infected with Chagres and/or Arumowot viruses, Gabek Forest infection was less severe, indicating some degree of cross-protection. The degree of cross-protection was in part related to the sequence of previous phlebovirus infections. No evidence of immune enhancement or other immunopathologic events were observed in the animals.

H. Summary

In conclusion, results of our studies indicate that a number of phleboviruses can be transovarially transmitted and that some of these agents can be maintained in their insect vectors for several consecutive generations by vertical transmission. The ability of some sandfly species to produce eggs autogenously appears to further enhance virus survival by this mechanism. However, our results also indicate that these viruses cannot be maintained indefinitely in sandflies by transovarial transmission alone. The available data suggest that occasional amplification in a vertebrate host is also a necessary part of the natural transmission cycle of these agents. Phleboviruses apparently use both vertical transmission in their vector and amplification in a vertebrate host to insure their survival. These findings have important epidemiologic significance, since they indicate that phleboviruses can be maintained for prolonged periods of time even in the absence of adult vectors or of susceptible vertebrate hosts.

I. Publications Resulting From This Project

1. Tesh, R.B. and Modi, G.B.: Growth and transovarial transmission of Chandipura virus (Rhabdoviridae: Vesiculovirus) in Phlebotomus papatasi. **Am. J. Trop. Med. Hyg. 32:** 621-623, 1983.

2. Tesh, R.B. and Modi, G.B.: Development of a continuous cell line from the sand fly, Lutzomyia longipalpis (Diptera: Psychodidae), and its susceptibility to infection with arboviruses. **J. Med. Ent. 20:** 199-202,1983.

3. Modi, G.B. and Tesh, R.B.: A simple technique for mass-rearing Lutzomyia longipalpis and Phlebotomus papatasi (Diptera: Psychodidae) in the laboratory. **J. Med. Ent. 20:** 568-569, 1983.

4. Endris, R.G., Tesh, R.B. and Young, D.G.: Transovarial transmission of Rio Grande virus (Bunyaviridae: Phlebovirus) by the sand fly, Lutzomyia anthophora. **Am. J. Trop. Med. Hyg. 32:** 862-864, 1983.

5. McMahon Pratt, D., Modi, G. and Tesh, R.B.: Leishmania mexicana stage specific antigens found on promastigotes of infected Lutzomyia longipalpis. **Am. J. Trop. Med. Hyg. 32:** 1268-1271, 1983.

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Table 1

Growth of selected arboviruses in the LL-5 cell line

Virus identification	Strain	Taxonomic group (genus)	Titer*	Arthropod association
vesicular stomatitis (Indiana)	VP-98F	Vesiculovirus	7.5	sandfly
Chandipura	I653514	"	6.5	sandfly
Isfahan	91025-C	"	5.4	sandfly
Jurona	BeAr40578	"	5.9	mosquito
Poona 733646	Poona 733646	Rhabdovirus (unclassified)	6.1	sandfly
Kununurra	Or 194	"	5.6	mosquito
Klamath	M 1056	"	1.4	unknown
Changuinola	BT-436	Orbivirus	6.9	sandfly
Colorado tick fever	Florio	"	1.4	tick
Kemerovo	EgAn 1169-61	"	4.3	tick
St. Louis encephalitis	Porton	Flavivirus	3.5	mosquito
West Nile	Egypt 101	"	4.3	mosquito and tick
Chikungunya	Ross	Alphavirus	1.9	mosquito
Ross River	T 48	"	1.7	mosquito
Cache Valley	Holden	Bunyavirus	1.4	mosquito
Naples sandfly fever	Naples	Phlebovirus	1.4	sandfly
Sicilian sandfly fever	Sicilian	"	1.4	sandfly
Punta Toro	D-40210A	"	1.4	sandfly
Chagres	JW 10	"	1.4	unknown
Gabek Forest	SudAn 754-61	"	5.7	unknown
Pacui	BeAn 27326	"	1.4	sandfly
Anhanga	BeAn 46852	"	4.6	unknown
Icoaraci	BeAn 24262	"	3.5	sandfly and mosquito
Arumowat	Ar 1284-64	"	1.4	mosquito
Joa	BeAr 371637	"	1.4	sandfly
Aguacate	VP-175A	"	1.4	sandfly
Salehabad	I-81	"	1.4	sandfly
Candiru	BeH 22511	"	1.4	unknown
Karimabad	I-58	"	1.4	sandfly

*Virus titer expressed as $\log_{10}$ of plaque forming units (PFU) on the fifth day after inoculation with $10^{2.0}$ PFU.

Table 2

Growth of Rio Grande virus in Lutzomyia anthophora after intrathoracic inoculation

Day post-inoculation	Range of titers in positive flies*	Mean titer of positive flies*
0	0.4 - 1.1	0.6
1	0.7 - 1.7	1.3
2	1.7 - 3.4	2.5
3	1.7 - 3.7	2.6
4	2.9 - 3.1	3.1
5	3.4 - 5.0	4.1
6	NT	NT
7	4.3 - 5.7	5.0
8	4.0 - 4.3	4.2

*Virus titers expressed as $\log_{10}$ of tissue culture infectious $dose_{50}$ per insect. Five sandflies were sampled each day.

Table 3

Growth of Belterra virus in Lutzomyia longipalpis after intrathoracic inoculation

Day post-inoculation	Range of virus titers in in infected flies*	Mean virus titer*
0	3.0 - 3.7	3.3
1	2.5 - 2.7	2.6
2	2.8 - 3.2	3.1
3	2.8 - 3.6	3.4
4	2.0 - 3.0	2.6
5	2.6 - 3.4	2.8
6	3.2 - 3.4	3.3
7	2.6 - 3.7	3.3

*Virus titers expressed as $\log_{10}$ of plaque units per insect. Five sandflies were sampled each day.

Table 4

Growth of Pacui virus in Phlebotomus papatasi after intrathoracic inoculation

Day post-inoculation	Range of titers in positive flies*	Mean titers in positive flies
0	<0.7 - 1.7	-
1	1.7 - 3.5	2.7
2	3.0 - 4.6	4.0
3	4.5 - 4.7	4.6
4	4.5 - 5.5	4.8
5	4.3 - 4.8	4.6
6	4.0 - 5.0	4.5
7	3.8 - 5.0	4.4

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sandflies were sampled each day.

Table 5

Development of Naples virus in Lutzomyia longipalpis after intrathoracic inoculation

Day post-inoculation	Virus titers in sand flies sampled*
0	2.4, 2.4, 2.7, 2.7, 2.8
1	<0.7, 1.3, 1.4, 1.4
2	1.3, 1.5, 1.5, 1.7, 1.8
3	<0.7, 0.7, 1.2, 1.3, 1.6
4	<0.7, 2.2, 2.3, 2.6, 2.6
5	2.2, 2.3, 2.3, 2.3, 2.4
6	2.2, 2.6, 2.6, 2.8, 3.2
7	<1.7, <1.7, 2.2, 2.3, 3.2

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect.

Table 6

Growth of Punta Toro virus in Phlebotomus papatasi after intrathoracic inoculation

Day post-inoculation	Virus titer in inoculated insects*
0	0.7, 0.7, 1.4, 1.5, 1.7
1	<0.7,<0.7,<0.7, 0.7, 0.7
2	<1.7,<1.7, 2.0, 2.0, 2.7
3	<1.7,<1.7, 2.2, 2.3, 2.7
4	2.0, 2.6, 2.7, 2.8, 3.0
5	<1.7,<1.7, 1.7, 1.7, 2.5
6	3.2, 3.6, 3.6, 3.6, 3.7
7	<1.7,<1.7, 2.0, 2.2, 2.7

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sandflies were sampled each day.

Table 7

Growth of Karimabad virus in Phlebotomus papatasi after intrathoracic inoculation

Day post-inoculation	Range of titers in positive flies*	Mean titer of positive flies*
0	2.0 - 2.6	2.2
1	2.5 - 2.8	2.6
2	3.6 - 4.5	4.2
3	3.4 - 4.5	3.9
4	4.0 - 5.2	4.6
5	4.0 - 4.6	4.4
6	4.0 - 4.8	4.3
7	4.0 - 4.5	4.3
8	4.0 - 4.8	4.3

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sandflies were sampled each day.

Table 8

Growth of Sicilian virus in Phlebotomus papatasi after intrathoracic inoculation

Day post-inoculation	Range of titers in positive flies*	Mean titer of positive flies*
0	<1.0	<1.0
1	<1.7 - 2.0	1.8
2	2.0 - 4.0	3.0
3	3.7 - 4.7	4.4
4	3.2 - 5.4	4.0
5	3.5 - 4.2	3.9
6	3.6 - 4.7	4.3
7	3.5 - 4.8	4.3

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sandflies were sampled each day.

Table 9

Growth of Saint Floris in Phlebotomus papatasi after intrathoracic inoculation

Day post-inoculation	Range of titers in positive flies*	Mean titers in positive flies*
0	2.0 - 2.6	2.4
1	1.7	1.7
2	2.8 - 3.7	3.2
3	3.0 - 4.2	3.5
4	3.0 - 3.8	3.4
5	3.6 - 4.0	3.8
6	4.0 - 4.3	4.1
7	3.6 - 4.3	3.9

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sandflies were sampled each day.

Table 10

Growth Of Rift Valley Fever Virus In Phlebotomus Papatasi After Intrathoracic Inoculation

Day post inoculation	Number infected/ number sampled	Range of titers in infected flies*	Mean titer in infected flies*
0	10/10	1.7 - 2.7	2.2
1	10/10	2.4 - 4.2	3.2
2	10/10	4.0 - 5.6	4.7
3	10/10	4.7 - 5.6	5.3
4	10/10	4.8 - 5.6	5.4
5	10/10	4.5 - 5.6	5.4
6	10/10	4.6 - 5.7	5.2
7	4/4	5.2 - 5.6	5.4
14	7/7	4.8 - 5.7	5.0

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect.

Table 11

Growth Of Rift Valley Fever Virus In Phlebotomus Papatasi After Blood Feeding On A Viremic Hamster*

Day post blood feeding	Number infected/ number sampled	Range of titers in infected flies**	Mean titer in infected flies**
0	5/5	4.2 - 4.7	4.5
1	5/5	3.9 - 4.3	4.1
2	5/5	3.4 - 3.8	3.6
3	1/5	2.0	2.0
4	1/5	1.0	1.0
5	0/5	0	0
6	1/5	5.2	5.2
7	1/5	2.2	2.2
8	0/5	0	0

*Pre- and post-exposure blood samples in the infected hamster were $10^{8.1}$ and $10^{8.7}$ PFU/ml of blood, respectively.

** Titers expressed as $\log_{10}$ of plaque forming units per insect.

Table 12

Growth of Gabek Forest virus in Lutzomyia longipalpis after intrathoraic inoculation

Day post-inoculation	Range of titers in positive flies*	Mean titer of positive flies*
0	2.6 - 3.0	2.8
1	1.2 - 2.2	1.6
2	1.7 - 2.3	2.0
3	2.7 - 3.6	3.1
4	2.7 - 3.5	3.2
5	2.8 - 3.6	3.3
6	3.0 - 4.2	3.6
7	3.7 - 4.3	4.0

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sandflies were sampled each day.

Table 13

Growth of Itaituba virus in Lutzomyia longipalpis after intrathoracic inoculation

Day post-inoculation	Virus titers in insects sampled*
0	<0.7, 1.2, 1.3, 1.4, 1.7
1	<0.7, <0.7, 0.7, 0.7, 0.7
2	<0.7, <0.7, <0.7, <0.7, 0.7
3	1.7, 1.7, 2.6, 2.6, 3.7
4	2.5, 2.5, 3.0, 3.0, 3.3
5	<0.7, 2.4, 2.6, 2.9, 4.2
6	<0.7, 3.0, 3.6, 3.6, 3.7
7	2.7, 3.0, 3.2, 4.0, 4.5

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sandflies were sampled each day.

Table 14

Growth of Salehabad virus in Phlebotomus papatasi after intrathoracic inoculation

Day post-inoculation	Range of titers in positive flies*	Mean titer of positive flies*
0	2.8 - 4.0	3.6
1	3.5 - 3.6	3.6
2	3.3 - 3.9	3.6
3	3.2 - 3.6	3.4
4	3.0 - 3.6	3.4
5	2.9 - 3.4	3.1
6	3.0 - 4.0	3.3
7	3.0 - 3.5	3.2

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sandflies were sampled each day.

Table 15

Growth of Chandipura virus in Phlebotomus papatasi after intrathoracic inoculation

Day post-inoculation	Range of titers in positive flies*	Mean titer of positive flies*
0	1.2 - 2.0	1.5
1	5.0 - 5.8	5.4
2	5.2 - 6.4	5.6
3	4.6 - 5.3	5.0
4	3.8 - 4.8	4.4
5	4.3 - 4.9	4.6
6	3.6 - 5.2	4.5

*Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sand flies were sampled each day.

Table 16

Growth of Toscana virus in P. perniciosus, P. papatasi, and L. longipalpis after intrathoracic inoculation

Day post-inoculation	P. perniciosus		P. papatasi		L. longipalpis	
	Range of virus titers in pos. flies*	Mean virus titer	Range of virus titers in pos. flies*	Mean virus titer	Range of virus titers in pos. flies*	Mean virus titer
0	2.0 - 2.7	2.2	1.4 - 2.4	1.9	1.6 - 2.7	2.2
1	2.0 - 3.0	2.6	2.0 - 2.8	2.5	1.8 - 2.6	2.1
2	2.5 - 3.7	3.0	1.7 - 2.9	2.1	2.0 - 2.7	2.3
3	3.2 - 4.2	3.9	2.3 - 3.2	2.8	1.7 - 2.2	2.0
4	3.2 - 4.7	3.7	2.5 - 3.3	2.9	2.2 - 2.6	2.4
5	3.0 - 4.2	3.6	2.4 - 3.0	2.8	1.7 - 2.6	2.4
6	3.8 - 4.7	4.2	2.0 - 3.0	2.6	2.5 - 3.5	2.9
7	3.0 - 4.0	3.7	-	-	2.4 - 3.2	2.6

* Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sand flies were sampled each day.

Table 17

Comparative growth of Arbia virus in Phlebotomus perniciosus following intrathoracic inoculation and ingestion

Day post-infection	Virus titers in inoculated females	Virus titers in fed females*
0	1.2,1.2,1.4,1.6,1.6**	2.7,2.8,2.8,2.8,3.2
1	<1.7,<1.7,<1.7,<1.7,<1.7	2.0,2.0,2.4,2.8,
2	2.2,2.5,3.0,3.0,3.4	(Not Tested)
3	3.0,3.0,3.0,3.3,3.8	<0.7,<0.7,<0.7,<0.7,<0.7
4	2.0,3.0,3.4,3.5,3.7	<0.7,<0.7,<0.7,<0.7,<0.7
5	3.7,3.7,4.0,4.2,4.4	<0.7,<0.7,<0.7,<0.7,<0.7
6	2.5,3.2,3.6,3.7,	<0.7,<0.7,<0.7,<0.7,<0.7

* Insects were fed on a mixture of infected mouse brain and washed human erythrocytes.

** Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sand flies were sampled each day.

Table 18

Comparative growth of Naples virus in Phlebotomus papatasi following intrathoracic inoculation and ingestion

Day post-infection	Virus titers in inoculated females	Virus titers in fed females*
0	2.0,2.0,2.0,2.5,2.7**	2.0, 2.6, 2.6, 2.9, 3.2
1	2.0,2.2,2.2,2.4,2.6	1.7, 2.0, 2.6, 2.6, 2.8
2	2.8,2.9,3.0,3.3,3.4	<0.7, 1.0, 2.2, 2.2, 2.3
3	3.0,3.5,3.6,4.2,4.2	<0.7,<0.7, 0.7, 0.7, 1.2
4	3.0,3.0,3.3,3.7,4.3	<0.7,<0.7,<0.7,<0.7,<0.7
5	3.0,3.4,3.6,4.0,4.0	<0.7,<0.7,<0.7,<0.7,<0.7
6	4.0,4.0,4.0,4.2,4.2	<0.7,<0.7,<0.7,<0.7,<0.7
7	3.8,4.0,4.2,4.4,4.5	<0.7,<0.7,<0.7,<0.7,<0.7

* Insects were fed on a mixture of infected newborn mouse brain and washed human erythrocytes.

** Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sand flies were sampled each day.

Table 19

Comparative growth of Punta Toro virus in Lutzomyia longipalpis following inoculation and ingestion

Day post-infection	Virus titers in inoculated females	Virus titers in fed females*
0	2.0,2.0,2.2,2.3,2.3**	2.4, 2.8, 2.8, 3.9, 4.2
1	1.7,1.7,2.0,2.9,3.2	2.0, 2.8, 3.0, 3.0, 3.3
2	3.0,3.0,3.4,3.6,3.8	<0.7,<0.7,<0.7, 0.7, 0.7
3	3.9,4.0,4.0,4.2,4.2	<0.7,<0.7, 0.7, 1.3, 1.7
4	3.7,3.7,4.0,4.4,4.4	<0.7,<0.7,<0.7,<0.7, 0.7
5	3.5,3.9,4.0,4.3,4.6	<0.7, 0.7, 1.0, 1.0, 1.4
6	4.0,4.2,4.2,4.3,4.7	<0.7,<0.7,<0.7,<0.7, 0.7
7	4.0,4.3,4.6,4.6,4.6	(Not tested)

* Sandflies were fed on a hamster infected with Punta Toro virus. Titer of hamster's viremia = $10^{7.8}$ PFU/ml.

** Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sand flies were sampled each day.

Table 20

Comparative growth of Pacui virus in Lutzomyia longipalpis following intrathoracic inoculation and ingestion

Day post-infection	Virus titers in inoculated females	Virus titers in fed females*
0	2.0,2.2,2.4,2.5,2.8**	3.2,3.4,3.5,3.6,3.9
1	4.0,4.0,4.0,4.0,4.8	<0.7,<0.7,<0.7,<0.7,<0.7
2	4.6,4.7,5.0,5.2,5.4	<0.7,<0.7,<0.7,<0.7, 0.7
3	5.2,5.2,5.4,5.5,5.6	<0.7,<0.7, 0.7, 1.0, 1.7
4	4.8,5.0,5.4,5.5,5.7	(Not tested)
5	5.0,5.2,5.2,5.3,5.4	<0.7,<0.7,<0.7,<0.7,<0.7
6	5.0,5.2,5.2,5.3,5.5	<0.7,<0.7,<0.7, 3.0, 3.4
7	4.0,4.8,5.0,5.0,5.6	<0.7,1.6,3.4,3.8
8	4.9,5.0,5.0,5.4,5.4	(Not tested)

* Insects were fed on a mixture of infected newborn mouse brain and washed human erythrocytes.

** Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sand flies were sampled each day.

Table 21

Comparative growth of Toscana virus in Phlebotomus perniciosus following inoculation and ingestion

Day post-infection	Virus titers in inoculated females	Virus titers in fed females*
0	2.0,2.0,2.2,2.2,2.7**	3.2,3.4,3.5,3.6,3.8
1	2.0,2.3,2.5,3.0,3.0	2.9,3.4,3.7,3.7,3.9
2	2.5,2.9,3.0,3.2,3.7	1.7,1.8,1.9,2.3,3.0
3	3.2,4.0,4.0,4.2,4.2	1.3,1.5,2.0,2.0,2.8
4	<1.7,2.8,3.2,4.2,4.7	<0.7,<0.7,<0.7, 1.0,3.0
5	3.0,3.3,3.7,4.0,4.2	<0.7,<0.7,<0.7, 2.7,2.8
6	3.8,4.0,4.0,4.3,4.7	2.8,2.9,3.0,3.2,3.7
7	3.0,3.8,3.8,3.9,4.0	<0.7,3.5,3.6,4.0

* Insects were fed on a mixture of infected mouse brain and washed human erythrocytes.

** Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sand flies were sampled each day.

Table 12

Comparative growth of Gabek Forest virus in Phlebotomus papatasi following inoculation and ingestion

Day post-infection	Virus titers in inoculated females	Virus titers in fed females*
0	1.0,1.7,2.2,2.2,2.5**	4.3,4.5,4.5,4.8,4.8
1	2.3,2.6 (only 2 tested)	4.2,4.5,4.9,5.0,5.0
2	2.7,3.0,3.2,3,2	<0.7,<0.7, 1.0,3.0
3	4.0,4.0,4.0,4.2,4.5	<0.7,<0.7,<0.7,2.4,3.7
4	4.0,4.8,5.0,5.0,5.5	<0.7,<0.7, 2.9,3.7,4.9
5	4.6,4.8,4.9,5.0,5.2	<1.7,2.0,2.0,3.2,3.7
6	4.6,4.9,5.2,5.3,5.3	<1.7,<1.7,<1.7,<1.7,<1.7
7	4.8,5.0,5.0,5.2,5.3	<1.7,<1.7,2.5,4.0,4.2

* Insects were fed on a hamster infected with Gabek Forest virus. Titer of hamster's blood = $10^{9.0}$ PFU/ml.

** Virus titers expressed as $\log_{10}$ of plaque forming units per insect.

Table 23

Growth of Arboledas virus in Lutzomyia gomezi after feeding on infected opossum*

Day post-feeding	Virus titer per insect sampled**
0	1.6, 1.6, 1.6, 1.7, 2.0
1	1.2, 1.3, 1.3, 1.4, 1.5
2	<0.7, 1.7, 2.0, 2.0, 2.0
3	1.6, 2.0, 2.0, 2.6, 3.0
4	2.2, 2.3, 2.7, 3.0, 3.4
5	3.0, 3.2, 3.4, 3.5, 3.6
6	3.9, 4.0, 4.0, 4.2, 4.2
7	4.2, 4.3, 4.4, 4.4

*Flies were fed on an opossum (D. virginiana) with a blood virus titer of $10^{5.6}$ PFU/mL.

**Five female flies were sampled daily. Virus titers expressed as $\log_{10}$ of PFU per insect.

Table 24

Comparative growth of Carajas virus (Rhabdoviridae: Vesiculovirus) in Lutzomyia longipalpis following inoculation and ingestion

Day post-infection	Virus titers in inoculated females	Virus titers in fed females*
0	1.4,1.7,1.8,1.8,1.9**	3.0,3.0,3.2,3.3,3.4
1	3.0,3.3,3.4,3.5,3.6	2.3,2.6,2.6,2.7,2.7
2	2.8,3.8,3.8,3.9,4.0	<0.7, 1.2,1.2,1.2,1.4
3	3.3,3.8,4.0,4.0,4.4	<0.7,<0.7,<0.7,<0.7,3.0
4	4.3,4.8,5.0,5.0,5.3	<0.7,<0.7,<0.7,<0.7,<0.7
5	4.6,5.0,5.0,5.0,6.0	<0.7,<0.7,<0.7,<0.7,<0.7
6	4.4,4.9,5.2,6.0,6.2	<0.7,<0.7,<0.7,<0.7,<0.7
7	4.0,4.5,4.5,4.9,5.2	<0.7,<0.7,<0.7,<0.7,<0.7

* Insects were fed on a mixture of infected mouse brain and washed human erythrocytes.

** Virus titers expressed as $\log_{10}$ of plaque forming units per insect. Five sand flies were sampled each day.

Table 25

Transovarial transmission rates of selected phleboviruses in sandflies

Virus used to infect parents*	Sandfly species	Number of F_1 progeny tested	Percentage of F_1 progeny infected
Rio Grande	Lutzomyia anthophora	62	54.8
Pacui	Lutzomyia longipalpis	122	32.0
Pacui	Phlebotomus papatasi	51	2.0
Sicilian	Phlebotomus papatasi	135	1.5
Gabek Forest	Phlebotomus papatasi	50	0
Punta Toro	Lutzomyia longipalpis	100	0
Karimabad	Phlebotomus papatasi	220	60.0
Rift Valley fever	Phlebotomus papatasi	235	0
Saint Floris	Phlebotomus papatasi	112	6.3
Salehabad	Phlebotomus papatasi	94	0
Toscana	Phlebotomus perniciosus	266	40.2
Toscana	Phlebotomus papatasi	117	0
Toscana	Lutzomyia longipalpis	240	0
Arbia	Phlebotomus perniciosus	87	20.7
Belterra	Lutzomyia longipalpis	60	0
Naples	Phlebotomus papatasi	179	0
Arboledas	Lutzomyia gomezi	31	80.6

*Female parents in these experiments were infected by inoculation.

Table 26

Vertical transmission of Toscana virus in chronically infected Ph. perniciosus line

Generation	Percentage infected	(Number /Total) (infected/tested)
Parental	100*	(25/25)
	↓	
F_1	40.2	(107/266)
	↓	
F_2	43.8	(35/80)
	↓	
F_3	82.5	(33/40)
	↓	
F_4	45.4	(54/119)
	↓	
F_5	86.0	(74/86)
	↓	
F_6	72.0	(54/75)
	↓	
F_7	61.8	(285/461)
	↓	
F_8	48.3	(42/87)
	↓	
F_9	33.3	(15/45)
	↓	
F_{10}	NT	
	↓	
F_{11}	14.1	(19/135)
	↓	
F_{12}	5.8	(7/119)
	↓	
F_{13}	57.9	(11/19)

* Female sand flies in the parental generation were experimentally infected by inoculation. In all subsequent generations, infection resulted from transovarial virus transmission.

NT = F_{10} generation sand flies were not tested.

Table 27

Toscana virus filial infection rates among F_1 generation offspring from individual Ph. perniciosus females

Female number	Number of progeny/Total infected /offspring tested	Filial infection rate (%)
1	13/21	61.9
2	7/16	43.7
3	23/27	85.2
4	15/27	55.6
5	23/35	65.7
6	20/27	74.1
7	3/5	60.0
8	12/24	50.0
9	12/22	54.5
10	6/16	37.5
11	8/9	88.9
12	23/27	85.2
13	17/25	68.0
14	12/13	92.3
15	9/19	47.4

Table 28

Toscana virus infection rates among F_7 generation transovarially infected male and female Ph. perniciosus adults

Date of eclosion	Males		Females	
	Number infected/tested	(Percentage infected)	Number infected/tested	(Percentage infected)
March 10-12	10/13	(76.9)	--	--
March 13-15	8/20	(40.0)	12/18	(66.7)
March 16-17	18/24	(75.0)	14/25	(56.0)
March 18-20	17/23	(73.9)	17/25	(68.0)
March 21-22	15/25	(60.0)	18/25	(72.0)
March 23-25	16/25	(64.0)	12/25	(48.0)
March 26-28	9/16	(56.3)	12/20	(60.0)
March 29 - April 2	5/8	(62.5)	10/15	(66.7)
TOTAL	98/154	(63.6)	95/153	(62.1)

Table 29 (Experiment 1)

Viremia in hamsters following infection with Gabek Forest virus*

	Virus titer in blood post-inoculation**			
Animal number	day 1	day 2	day 3	day 4
2206	0	7.8	9.0	(D)
2207	0	7.7	9.0	(D)
2252	0	8.3	(D)	-
2253	0	8.8	9.2	(D)
2254	0	8.0	9.6	(D)
2255	0	7.6	(D)	-

*Hamsters received $10^{4.6}$ PFU of Gabek Forest virus subcutaneously.
**Virus titer expressed as $\log_{10}$ of PFU/ml. 0= $10^{1.7}$ PFU/ml.
(D) Animal died.

Table 30 (Experiment 2)

Viremia and neutralizing antibody response in hamsters infected sequentially with Arumowot, Chagres and Gabek Forest viruses*

Animal number	Virus titer in blood post-inoculation** day 1	day 2	day 3	day 4	day 5	Neutralizing antibody titer 3 weeks post-inoculation *** AMT	CHG	GF
A. First infection (Arumowot)								
2229	3.0	6.0	6.7	3.2	0	320	10	10
2231	3.9	5.7	7.0	2.8	0	640	10	10
2234	3.4	6.0	6.2	3.6	0	2560	10	10
2235	5.2	6.2	6.5	1.7	0	160	10	10
2261	2.0	5.5	6.2	2.6	0	160	10	10
2262	0	5.0	6.3	2.7	0	80	10	10
B. Second infection (Chagres)								
2229	0	4.3	5.6	2.0	0	1280	320	10
2231	0	4.2	4.0	0	0	1280	320	10
2234	0	0	0	4.8	3.0	2560	160	10
2235	0	0	4.7	3.6	0	1280	320	10
2261	0	0	5.9	5.3	0	2560	1280	10
2262	0	0	6.0	5.4	0	160	80	10
C. Third infection (Gabek Forest)								
2229	-	-	-	0	-	320	160	40
2231	-	-	-	0	-	2560	320	160
2234	-	-	-	0	-	-	-	-
2235	-	-	-	(D)	-	-	-	-
2261	-	3.0	4.6	-	-	2560	1280	160
2262	-	0	0	-	-	320	1280	40

*Hamsters received $10^{5.4}$ PFU of Arumowot, $10^{3.0}$ PFU of Chagres, and $10^{4.6}$ PFU of Gabek Forest viruses subcutaneously.
**Virus titer in blood expressed as $\log_{10}$ of PFU/ml. 0= $10^{1.7}$ PFU/ml.
***Reciprocal of highest serum dilution producing 90% plaque reduction.
- No serum sample taken or tested.
(D)Hamster died.

Table 31(Experiment 3)

Viremia and neutralizing antibody response in hamsters infected sequentially with Chagres, Arumowot and Gabek Forest viruses*

	Virus titer in blood post-inoculation**					Neutralizing antibody titer 3 weeks post-inoculation ***		
Animal number	day 1	day 2	day 3	day 4	day 5	AMT	CHG	GF
A. First infection (Chagres)								
2236	0	5.6	5.9	0	0	10	2560	10
2237	0	2.2	6.0	5.2	0	10	160	10
2238	0	0	5.2	5.4	0	10	640	10
2241	0	0	0	2.5	5.6	10	2560	10
2263	0	3.6	5.5	5.0	0	10	320	10
2264	0	0	5.0	0	0	10	320	10
2265	0	0	4.5	5.3	0	10	320	10
2270	0	4.4	5.8	0	0	10	640	10
B. Second infection (Arumowot)								
2236	2.3	5.0	6.2	3.5	2.5	-	-	-
2237	2.0	5.3	6.0	3.3	2.2	40	320	10
2238	0	4.9	5.4	2.6	2.2	40	320	10
2241	0	4.4	6.0	0	0	640	2560	10
2263	0	3.2	4.0	5.6	0	40	320	10
2264	2.2	4.0	4.7	4.2	0	40	320	10
2265	2.2	4.8	6.6	5.0	0	80	640	10
2270	0	4.7	6.6	4.8	0	160	160	10
C. Third infection (Gabek Forest)								
2237	-	-	-	7.0(D)	-	-	-	-
2238	-	-	-	5.4	-	320	1280	80
2241	-	-	-	4.8	-	640	320	640
2263	-	5.6	7.8	-	(D)	-	-	-
2264	-	7.0	8.8	-	(D)	-	-	-
2265	-	5.0	6.0	-	-	320	640	80
2270	-	5.2	6.8	-	-	1280	640	320

*Hamsters received $10^{3.0}$ PFU of Chagres, $10^{5.4}$ PFU of Arumowot, and $10^{4.6}$ PFU of Gabek Forest viruses su

Table 32(Experiment 4)

Viremia and neutralizing antibody response in hamsters infected sequentially with Arumowot and Gabek Forest viruses*

Animal number	Virus titer in blood post-inoculation** day 1	day 2	day 3	day 4	day 5	Neutralizing antibody titer 3 weeks post-inoculation *** AMT	CHG	GF
A. First infection (Arumowot)								
2239	3.3	5.7	6.0	3.4	2.2	640	10	10
2219	3.6	4.7	3.9	2.3	0	80	10	10
2256	3.2	4.7	6.3	3.0	0	160	10	10
2257	2.6	6.0	6.8	3.4	0	160	10	10
2259	2.0	4.4	6.3	0	0	160	10	10
2266	0	5.6	6.9	5.6	2.7	160	10	10
2268	0	6.6	6.3	5.3	0	40	10	10
2269	0	5.7	6.9	5.7	0	80	10	10
B. Second infection (Gabek Forest)								
2239	-	-	-	7.0	- (D)+	-	-	-
2219	-	9.5 (D)	-	-	-	-	-	-
2256	-	5.3	5.8	-	-	320	10	160
2257	-	3.8	6.3	-	- (D)+	-	-	-
2259	-	5.2	5.7	-	-	160	10	40
2266	-	8.5	9.5 (D)	-	-	-	-	-
2268	-	7.2	9.0	(D)	-	-	-	-
2269	-	9.0	(D)	-	-	-	-	-

*Hamsters received $10^{5.4}$ PFU of Arumowot and $10^{4.6}$ PFU of Gabek Forest viruses subcutaneously.
**Virus titer in blood expressed as $\log_{10}$ of PFU/ml. 0 = $10^{1.7}$ PFU/ml.
***Reciprocal of highest serum dilution producing 90% plaque inhibition.
- No serum sample taken or tested.
(D)Hamster died.
+ Hamster #2239 and 2257 died 6 and 7 days respectively, after infection with Gabek Forest virus.

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